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Sunlight and Melanin Pigmentation*

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Madhu A. Pathak, Kowichi Jimbow, George Szabo, and Thomas B Fitzpatrick

Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114

1. INTRODUCTION

When viewed from the perspective of photobiology, melanin pigmentation of human skin can be described in two categories: the first, *constitutive* or *intrinsic skin color*, and the second, *facultative* or *inducible skin color* (Quevedo *et al.*, 1974). Constitutive skin color designates the genetically determined levels of cutaneous melanin pigmentation in accordance with the

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contains tyrosinase and (or) filaments that have a distinct 10-nm (100-Å) periodicity. The formation of melanosomes in this initial stage of development is regulated by the Golgi apparatus, the Golgi-associated endoplasmic reticulum, and rough endoplasmic reticulum. A stage I melanosome is believed to contain tyrosinase, structural proteins, membranes, and possibly certain auxiliary enzymes. It is speculated that polypeptides of the enzyme tyrosinase are synthesized on ribosomes and that the phospholipid-containing proteins are fashioned into membranes by the Golgi complex.

Stage II describes an oval organelle in which numerous membranous structures become evident, with or without cross-linking, and show a distinct 10-nm periodicity.

Stage III reflects an oval organelle in which melanin synthesis has begun through the enzymatic oxidation of tyrosine. Melanin accumulation occurs on the inner membrane, and the 10-nm periodicity evident earlier in stage II becomes partially obscured.

Stage IV reflects the end stage of development of melanosomes in which the deposition of melanin has resulted in the obliteration of the internal structure of melanosomes in stages II and III. The organelle is electron opaque and shows electron-lucent bodies at its periphery (Jimbow and Fitzpatrick, 1974*a*).

2.1.2. Melanization of Melanosomes

Melanin synthesis takes place within the melanosomes (Seiji *et al.*, 1963*a,b*; Fitzpatrick *et al.*, 1967; Moyer, 1966). The copper-containing enzyme tyrosinase, present in these organelles, catalyzes the oxidation of both monohydric (tyrosine) and dihydric phenols (3,4-dihydroxyphenylalanine) to orthoquinones. Molecular oxygen acts directly as the hydrogen acceptor in the reactions catalyzed by tyrosinase. In contrast to melanins of plant origin, which are generally described as *catechol melanin* in type, the mammalian melanins are *indole* in type and are composed basically of indole-5,6-quinone units. According to the classic Raper–Mason scheme of enzymic melanin formation from tyrosine and 3,4-dihydroxyphenylalanine (dopa) critically reviewed by Mason (1967), melanin is thought to be formed from tyrosine → dopa → dopa-quinone → dopa-chrome → 5,6-dihydroxyindole → indole-5,6-quinone → melanin through the polymerization of indole-5,6-quinone units (a homopolymer of indole-5,6-quinone linked through a single bond type). However, several chemical studies of natural melanins (Nicolaus, 1968; Swan, 1963, 1964; Robson and Swan, 1966; Hempel, 1966) using ^{3}H-labeled and ^{14}C-labeled precursors have revealed that mammalian melanins are not composed entirely of indole-5,6-quinone units, but are complex polymers (a heteropolymer or a random polymer) consisting of several different monomers that may be coupled by various bond types of dopa-quinone, dopa-chrome, 5,6-dihydroxyindole, and 5,6-dihydroxyindole-2-carboxylic acid at various oxidation levels held together by a variety of bond types. Pyrrole units, carrying carboxyl groups, in addition to phenolic or quinonoid groups or both, are also present, and some of them represent carboxylated terminal units. The redox state of melanin polymer is equally important. The polyquinonoid melanin can have oxidized (quinonoid), as well as reduced (phenolic) forms of indole-5,6-quinone. These remarks relate only to the synthesis of the black–brown melanin (eumelanin) and much less is known about the yellow and red pigments in mammals (phaeomelanins) which differ from eumelanin. Eumelanin is insoluble in almost all solvents and is resistant to chemical treatments, whereas pheomelanin is soluble in dilute alkali (Duchon *et al.*, 1968; Fitzpatrick *et al.*, 1971*a,b*). It is believed that dopa-quinone formed from the oxidation of tyrosine by tyrosinase interacts with the sulfur-containing amino acid, cysteine, to form cysteinyldopa from which phaeomelanin is derived by pathways yet incompletely understood (Fitzpatrick *et al.*, 1971*b*, Quevedo *et al.*, 1974).

2.1.3. Transfer and Dispersion of Melanosomes

We have discussed the fact that the epidermal melanocytes are in symbiotic relationship with the epidermal keratinocytes. The importance of this symbiotic relationship can be well recognized when one examines the distribution of melanosomes after their transfer into keratinocytes in various races (Mottaz and Zelickson, 1967; Klaus, 1969). Electron microscopic observations of human epidermis obtained from people of various racial backgrounds have revealed that melanosomes occur within keratinocytes as discrete granules (nonaggregated form) or as aggregates of two or more discrete granules within membrane-limited bodies (Hori *et al.*, 1968; Szabo *et al.*, 1969; Toda *et al.*, 1972; Wolff and Konrad, 1971; Olson *et al.*, 1973). In Caucasoids and Mongoloids, the melanosomes are almost always found in groups (Fig. 1) and resemble the membrane-limited vacuoles that have been identified as phagolysosomes (Mishima, 1967*a,b*; Hori *et al.*, 1968). In the melanosome complexes of fair-skinned Caucasoids, the melanosomes are loosely packed and there is some granular substance located between them. In Mongoloids and Orientals, the groups of melanosomes are usually very tightly packed, with little or no substance between the melanosomes. After UV irradiation, the keratinocytes of Mongoloids and Caucasoids usually still contain melanosome complexes

(Szabo *et al.*, 1969). However, the number of groups increases, and there is a tendency toward increased numbers of melanosomes inside the melanosome complexes. The keratinocytes of Negroids (African and American) and Australoids (Szabo *et al.*, 1969; Mitchell, 1968; Toda *et al.*, 1972) contain mostly single melanosomes, and only occasionally does one find groups and doublets of melanosomes inside keratinocytes (Fig. 1). This aggregation or nonaggregation of melanosomes in keratinocytes appears to be a size-dependent phenomenon (Toda *et al.*, 1972; Wolff and Konrad, 1971) inasmuch as ellipsoidal melanosomes smaller than 0.6 × 0.3 nm in size are usually arranged in groups of two or more and show evidence of degradation. Unlike those of Caucasoids and Mongoloids, the melanosomes of Negroids and Australian aborigines are larger (0.7–0.8 × 0.3–0.4 nm), and they usually do not form such aggregated complexes within the keratinocytes but are found as single, discrete bodies (Toda *et al.*, 1972; Olson *et al.*, 1973; Wolff and Konrad, 1971; Wolff *et al.*, 1974). Thus, variations in the size of melanosomes and the distribution pattern of melanosomes in the keratinocytes significantly influence the color of the skin. When melanosomes are aggregated and are few in number and small in size, they will contribute less to the scattering and absorption of impinging light than when they are singly dispersed, greater in number, and large in size (Table 1).

2.2. Normal Skin Color and Human Racial Color Differences

The most obvious difference between the various human races is the variation in the color of the skin. The factors that determine the skin color of normal skin include: (a) a reflection coefficient of skin surface; (b) absorption coefficient of epidermal-cell and dermal-cell constituents; (c) scattering coefficients of various cell layers; (d) thickness of the individual cell layers (stratum corneum, epidermis, and dermis); (e) the concentration of UV light and visible light absorbing components such as proteins (keratin, elastin, collagen, lipoprotein), melanin, nucleic acid, urocanic acid, carotenoids, hemoglobin (reduced and oxidized), and lipids; (f) the number and spatial arrangement of melanosomes and melanocytes; (g) the number and spatial arrangement of blood vessels and the relative quantity of blood cells (reduced and oxidized hemoglobin) flowing through the vessels. Pigmentation of the skin, as viewed clinically, is principally related to the variation in the content of melanin in the epidermis. If melanin pigment were absent from the skin, as in Vitiligo, the color of skin in all races would appear to be milk-white.

It appears therefore, that the color of skin is determined by the func-

TABLE 1. Relationship between Constitutive Skin Color and Size and Distribution Pattern of Melanosomes in Habitually Exposed Skin

Skin color	Size of melanosomes	Melanization of melanosomes	Tyrosinase activity in melanocytes	Distribution of melanosomes in epidermal keratinocytes	Approximate number of melanosomes per basal keratinocyte*
Heavily pigmented skin of African and American Negroes and Australian Aborigines	0.7–0.8 μm × 0.3–0.4 μm	Fully melanized, predominantly in stage IV	Marked	Single, non-aggregated	400 ± 35
Moderately pigmented skin of Mongoloids (American Indians, Orientals)	0.5–0.7 μm × 0.2–0.4 μm	Moderately melanized stages III and IV	Moderate	Mixed, non-aggregated as well as aggregated	250 ± 50
Moderately pigmented skin of Caucasoids (East Indians, Italians, Egyptians)	0.5–0.7 μm × 0.2–0.4 μm	Moderately melanized stages III and IV	Moderate	Predominantly aggregated	200 ± 5
Lightly pigmented skin of Caucasoids (fair-skinned Americans, British, French, Germans, etc.)	0.4–0.6 μm × 0.2–0.4 μm	Partially melanized stages II and III	Weak	Predominantly aggregated	100 ± 50

* Based on random calculations of 50 keratinocytes of basal layer.

tional state of pigment-producing cells, the melanocytes. One of the most obvious questions to ask is how the function of melanocytes is related to phenotypical coloration, a coloration determined genetically (constitutive) or produced by environmental factors (facultative). On the macroscopical level one can qualitatively assign color grades (e.g., black, brown, olive, moderately fair, very fair, freckled, etc.) based upon the quantity of melanin in the epidermis. At the level of light microscopy, when skin biopsies are examined, one can also distinguish the variations in skin color by the presence of more or less melanin in the skin (Gates and Zimmerman, 1953). The color of human skin derives from the visual impact of the total melanin content of the epidermis and is influenced by the reflection, absorption, and scattering of the impinging radiation on the surface of the skin. But the racial origin or the background of an individual cannot be ascertained by the mere visual color of the skin based on its melanin content. A representative of the Mediterranean race or an Asiatic Caucasoid may be labeled as "white" during winter but as "colored" by the end of a sunny summer. It is also true that neither a numerical count of melanocytes nor any other histologic characteristic as seen by light microscopy in the paraffin-embedded skin sections could reveal the racial origin of the skin specimens. The epidermal melanocyte system of various human races has been investigated by Staricco and Pinkus (1957), Szabo (1954, 1959, 1967*a,b*), Mitchell (1963), and Toda *et al.*, (1973). These investigators compared the melanocyte density of single representatives of various "colored" groups with the average melanocyte frequency of white Caucasians and observed that the colored races do not have more melanocytes than white Caucasians. Careful studies of human skin, particularly of the unexposed regions of the body, have revealed that racial differences in skin color are not due to differences in the number and distribution of melanocytes but are due to characteristic differences in the rate at which melanosomes are produced by melanocytes and transferred and distributed in keratinocytes (Fitzpatrick *et al.*, 1965, 1971*a,b*; Toda *et al.*, 1973; Pathak, 1967; Pathak *et al.*, 1971; Quevedo *et al.*, 1974).

Racial color differences can be recognized at the ultrastructural level and involve: (a) differences in the localization or distribution pattern of melanosomes in the keratinizing malpighian cells, either in the aggregated or in the nonaggregated form, or as a combination of aggregated and nonaggregated forms; (b) variation in the number of melanosomes in the epidermal melanocytes and keratinocytes; (c) differences in the size of melanosomes; (d) differences in the degree of melanization of melanosomes; and (e) differences in the degradation of melanosomes due to variations in the hydrolytic activity of these organelles. Variations in the hydrolytic activity of the melanosomes can influence the degradation of melanosomes. The

lighter skin color of Caucasoids may result from the degradation of mel nosomes, a phenomenon that has been observed to occur within ke atinocytes (Hori *et al.*, 1968; Szabo *et al.*; Toda *et al.*, 1974).

3. FACULTATIVE MELANIN PIGMENTATION: ACTION OF LIGHT

Solar radiation profoundly influences skin color. Increased melan pigmentation which occurs after exposure of human skin to sunlight or UV light from artificial sources is familiarly known as "tanning." Tannir of the skin involves two distinct photobiologic processes: (1) immediate tai ning (IT), sometimes referred to as immediate pigment darkening (IPI reaction, and (2) delayed tanning (DT) (Fig. 2). The biophysica biochemical, and ultrastructural bases of these two processes will be re viewed briefly with special emphasis on the effects of single and multiple ex posures to UV radiation on (a) changes in the number of melanocytes (Figs 3, 4); (b) synthesis of melanosomes, i.e., the number of melanosomes an their size; (c) melanization of melanosomes; and (d) the transfer of mela nosomes to keratinocytes; concomitant changes in keratinocytes concernin (e) the number melanosomes transferred; and (f) the distribution pattern o melanosomes within the keratinocytes.

3.1. Immediate Tanning Reaction

IT can best be seen in pigmented individuals or in the previously tan ned areas of fair-skinned individuals. IT can be induced both by long-wave UV (315–400 nm) and visible light (400–700 nm). UV-A (315–400-nm radiation is more effective in the induction of IT than is visible radiation (Pathak, 1967; Pathak *et al.*, 1962*a,b*; Pathak and Stratton, 1969; Jimbow and Fitzpatrick, 1975; Jimbow *et al.*, 1974*a,b*, 1975*a,b*). UV-B radiation (290–315 nm), the sunburn-producing spectrum, does not stimulate IT as effectively as UV-A radiation. This selective induction of IT by UV-A is related to the depth of penetration and absorption of this radiation at the dermo-epidermal junction. The skin begins to be hyperpigmented with 5–10 min of midday summer sun exposure and can be maximally pigmented with 1 h of irradiation. When the skin is withdrawn from exposure to light, the hyperpigmentation fades rapidly within 30–60 min, and thereafter the color usually fades gradually, so that after 3–4 h the irradiated areas are barely hyperpigmented. Sometimes, however, after prolonged sun exposure, 90–120 min, skin may remain hyperpigmented for as long as 36–48 hr, after which time newly synthesized melanin (new melanogenesis or DT) begins to ha

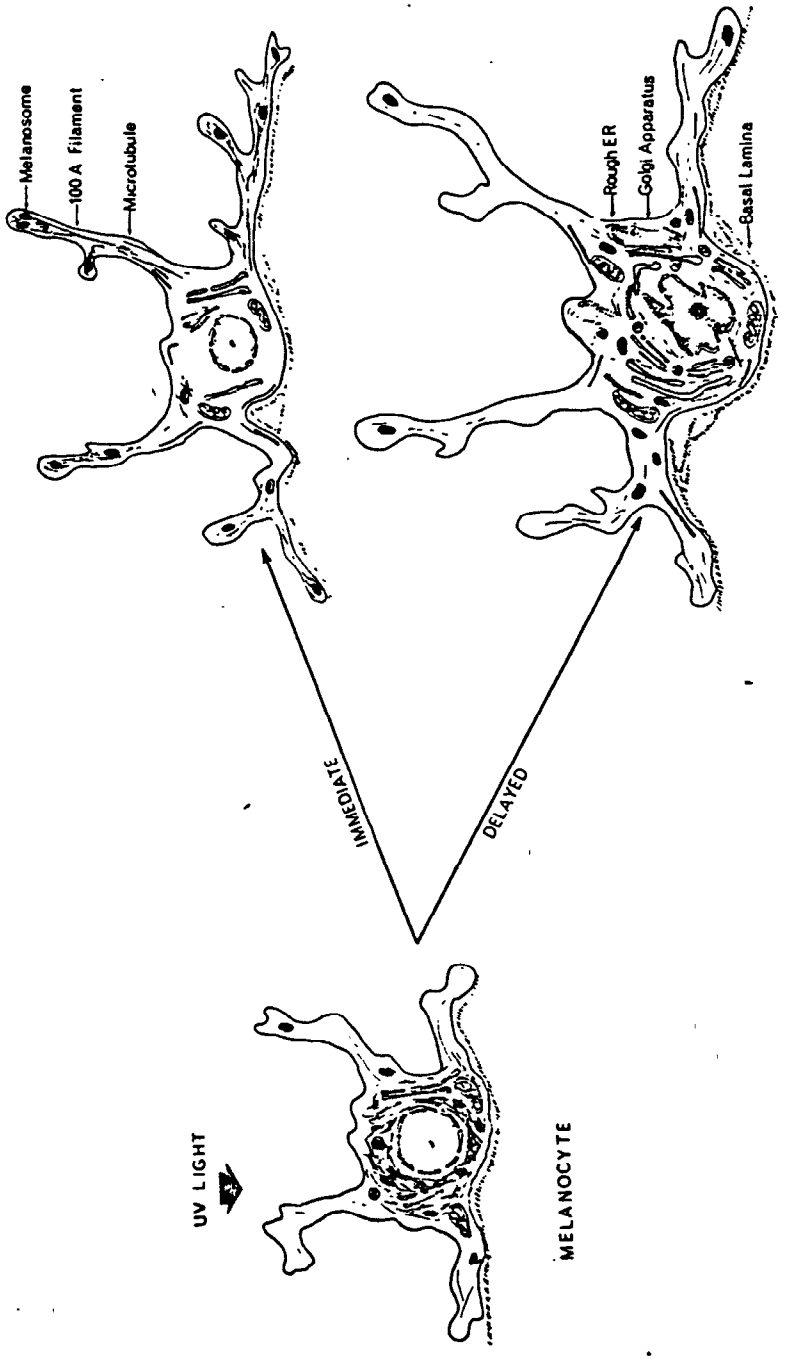

Fig. 2. Two photobiologic processes in melanin pigmentation stimulated by sunlight are illustrated: immediate tanning (IT) and delayed tanning (DT) reaction. The left side of the figure shows a melanocyte from the unexposed skin. This melanocyte shows a round or oval nucleus, few melanized melanosomes, and dense aggregates of fine filamentous structures around the nucleus. Top right shows a melanocyte after induction of IT. Melanocytic thin 10-nm filaments are hardly seen in the perinuclear area; they can be seen in the dendritic processes. The melanosomes are closely intermingled with 10-nm filaments. Lower right shows a hypertrophic melanocyte with well-developed dendrites after DT.

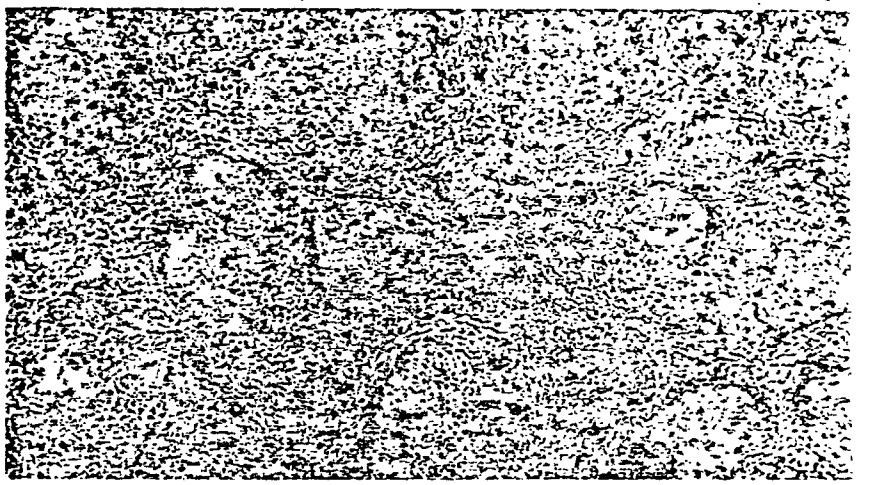

Fig. 3. A low-power view of dopa-incubated epidermal preparation from the unexpos Caucasoid skin. Biopsy was initially incubated in 2N NaBr, epidermis was then split from t dermis and reincubated in dopa solution (Starrico and Pinkus, 1957). The perikaryon of t melanocyte is small and the dopa reaction is weak.

perpigment the skin. The residual hyperpigmentation of IT is due to tl redistribution of the existing melanosomes within the keratinocytes. I asmuch as the IT reaction is a rapid phenomenon and can be induced in matter of a few minutes, it appears that IT results from changes in mel nosomes already existing in the melanocytes and keratinocytes of skin and brought about by a combination of several of the events described below.

3.1.1. Photooxidation of Preformed Melanin

An immediate photooxidation of already existing melanin polymer o curs through the generation of semiquinonelike free radicals in melan (Pathak, 1967; Pathak and Stratton, 1968). As stated earlier, the indole-5, quinone units in the melanin polymer can exist in different stages of oxid tion. The comparatively reduced state of melanin is evidenced by a brow or lightly tanned color of the skin, and the comparatively oxidized form ca be recognized as a dark brown or black color. One of the most importa properties of melanins is their stable, free radical character which is a cribed to the semiquinonoid form of 5,6-dihydroxyindole that is stabiliz by resonance throughout the highly conjugated polymer. The free radic content depends upon the degree of melanization and oxidation (Mason *al.*, 1960; Blois *et al.*, 1964; Pathak, 1967; Stratton and Pathak, 196 Pathak and Stratton, 1969). Prior to irradiation the lightly tanned sk

Fig. 4. A low-power view of dopa-incubated biopsy preparation at day 5 after exposure to UV radiation (150 mJ/cm², 290–320 nm). The intensity of the dopa reaction is markedly increased, the perikaryon of the melanocyte is hypertrophic, and there is marked arborization of the dendritic process.

exhibits a weak electron paramagnetic resonance signal characteristic of melanin free radicals. Immediately after irradiation with UV and visible light, the color of the skin changes to dark brown or black and one can detect a significant increase in the semiquinonelike free radicals in the melanin polymer, suggesting that an immediate oxidation reaction is also occurring in the polymer. Furthermore, the unexposed skin of fair individuals contains melanosomes which are partially melanized and can be recognized in stages II and III of their development. After induction of IT, however, one can often detect melanosomes in stages III and IV (highly melanized form). The unexposed skin of darkly pigmented individuals (e.g., Mongoloids, Negroids, and pigmented Caucasoids) already contains melanosomes in stages III and IV. Their melanosomes are mostly in stage IV when the IT reaction is induced.

3.1.2. Changes in the Distribution Pattern of Melanosomes in the Epidermis

In the melanocytes of unexposed skin, the melanosomes are usually aggregated around the nucleus and are rarely seen in the dendritic processes (Figs. 2, 5, 6). After the induction of IT, however, the melanosomes become prominent in the dendritic processes (Figs. 2, 7, 8; Jimbow *et al.*, 1973, 1974*a*). There is also a definite change in the number and dispersion pattern

of melanosomes in keratinocytes after the IT reaction. A random count of melanosomes in keratinocytes with nuclei located in the basal layer of four subjects showed a statistically significant increase in the number of melanosomes per keratinocyte after the IT reaction. These findings suggest that during the IT reaction there was a rapid transfer and redistribution of pigmented melanosomes from the melanocytes to the keratinocytes. It is equally possible that this redistribution and variations in the melanosomal number discussed above reflect changes in the sol-gel property of the cytoplasm and also an increment in the negative charge of the melanosomes due to the absorption of radiant energy.

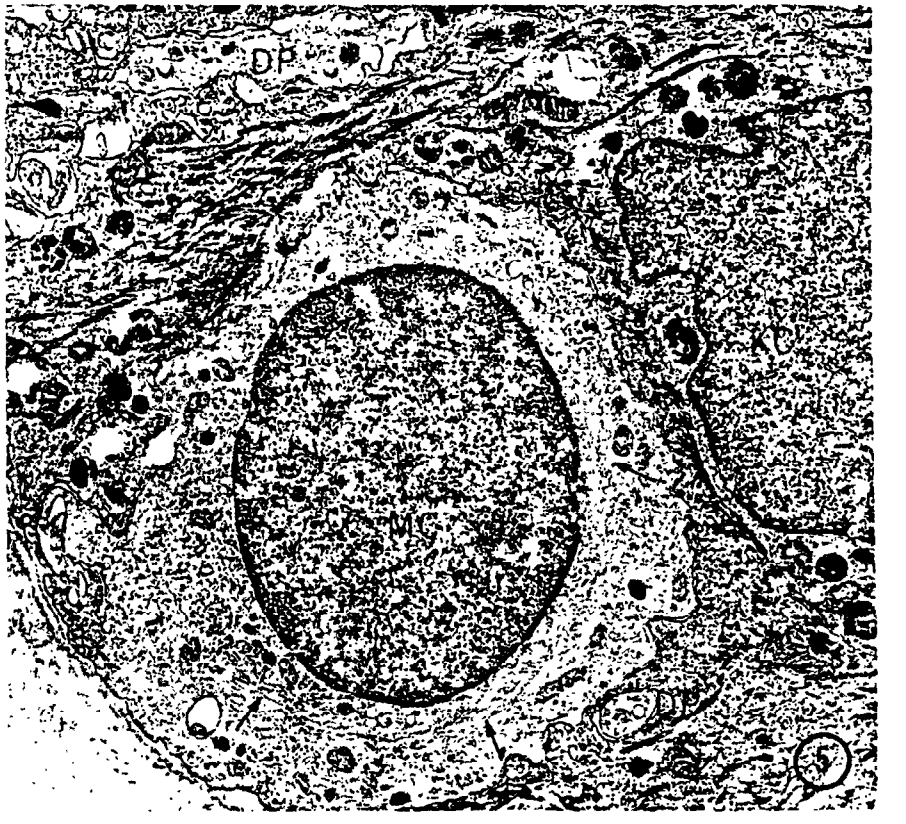

Fig. 5. An electron micrograph of unexposed skin of buttock from a Mongoloid subject. The melanosomes are mostly in melanizing stages (stages II and III). Dense aggregates of 10-nm filamentous structures (see arrows) around the oval nucleus can be seen. On the right side of the figure is a keratinocyte (KC); DP = dendritic processes, MC = melanocyte. (×10,200).

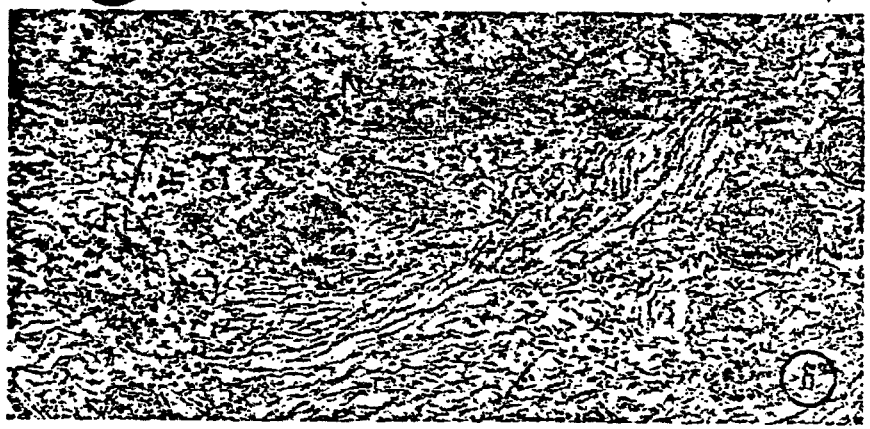

Fig. 6. A higher-power view of a portion of the melanocyte shown above. Around the nucleus, an aggregate of 10-nm filaments (F) and melanosomes in stages I–III can be seen. FL = fibrous lamina of the nucleus (N).

3.1.3. Changes in the Distribution Pattern of 10-nm Melanocytic Filaments

Recent observations of Jimbow and Fitzpatrick (1975*a*) and Jimbow *et al.* (1973, 1975*a*) have indicated that human melanocytes contain 10-nm-diameter filaments. These melanocytic filaments, and probably microtubules also, play a prominent role in the IT reaction and provide a motive force for rapid movement and transfer of melanosomes from melanocytes into the keratinocytes (Figs. 2–8). This hypothesis is based on the following observations. Prior to the induction of IT, the melanocytes from the habitually nonexposed regions of the body in the skin of Caucasoids, Mongoloids, and Negroids exhibit: (a) few melanosomes, (b) numerous 10-nm-diameter filaments, and (c) a few microtubules (25–27 nm in diameter) characteristically aggregated around the nuclei. *Per contra*, significant changes in the distribution pattern of these organelles in IT involved (a) prominence of dendritic processes laden with 10-nm-diameter filaments; (b) translocation of melanosomes from the perikaryon to dendritic processes which forms a concourse of melanosomes in the bundles and a meshwork of microfilaments; (c) a few microtubules in the extended processes of dendrites; and (d) a concomitant increase in the number of melanosomes in the keratinocytes (Jimbow and Fitzpatrick, 1975; Jimbow *et al.*, 1973, 1975*a*).

Thus, IT reflects changes in the existing melanosomes and does not involve the new synthesis of melanosomes. The most noticeable changes include (a) photooxidation of melanin, (b) marked change in the distribution pattern of melanocytic filaments and microtubules as well as melanosomes characterized by shifting and dispersion of these filaments and tubules from the perinuclear area to the dendritic processes of melanocytes, and (c) a

recognizable decrease in the number of melanosomes in the perikaryon : companied by an increase in the number of melanosomes in the k atinocytes.

3.2. Delayed Tanning Reaction and Hyperpigmentation of the Skin

Delayed tanning is a process which involves the production, transf distribution, and, to a limited extent, degradation of melanosomes (Jimb *et al.*, 1974*a,b*, 1975*b*). The degree of melanin pigmentation that occ following exposure of human skin to solar radiation varies to a cert

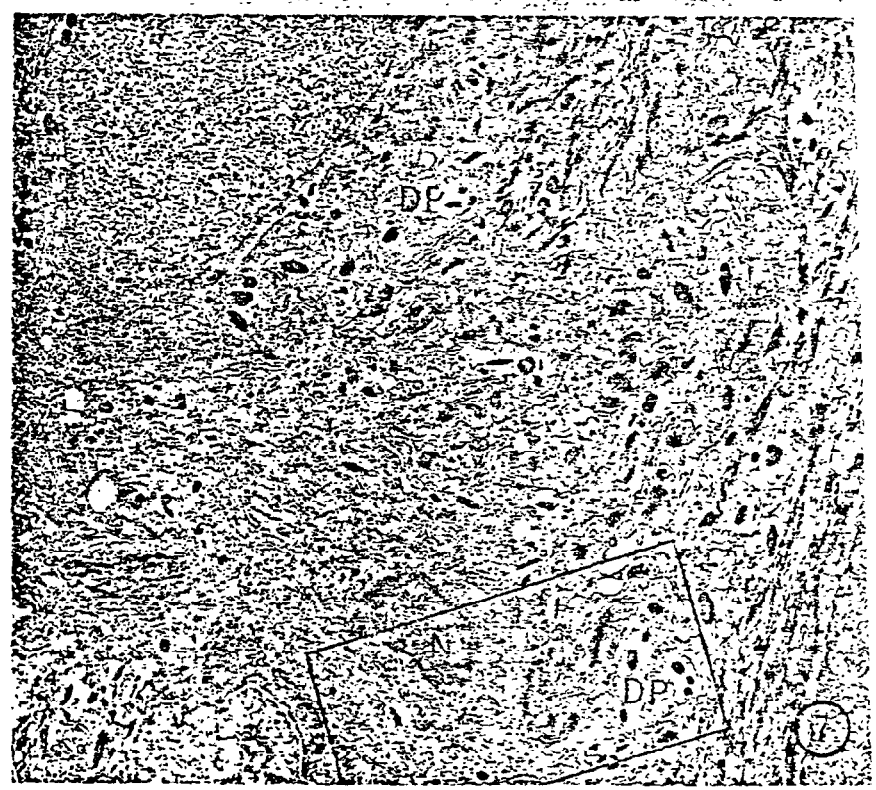

Fig. 7. A melanocyte from Mongoloid buttock skin after the IT reaction. The specimen wa taken from the same subject shown in Figs. 5 and 6. The dendritic processes (DP) of th melanocytes are well developed and extend into the keratinocytes. The melanosomes becom less aggregated in the perinuclear area (N = nucleus) and are now prominently seen in the dendritic process. The melanocyte contains more melanized melanosomes than those shown in Fig. 5 before exposure.

Fig. 8. High-power view of the portion of the melanocyte shown in a rectangular box in Fig. 7. The nucleus (N) is indented and shows nuclear pores. The 10-nm filaments appear to be stemming from perinuclear area to the tip of dendrites (see arrows). Microtubules (MT) are located in the periphery of the cytoplasm.

extent with the total dose of solar radiation received, but more importantly it is regulated by the genetically controlled functional capacity of the epidermal melanin unit of the individual. Genes control the structure of the melanosomes, the level of tyrosinase activity, the polymerization process of the indolequinone and other intermediates, and the development of the dendrites that transfer the melanosomes to the keratinocytes. Solar radiation or UV radiation from artificial sources influences the genetically controlled normal melanin pigmentation (facultative color) of the skin in one or more of the following ways: (a) an increase in the number of functional melanocytes (dopa-positive) as a result of proliferation of melanocytes, and also possibly the activation of the dormant or resting melanocytes (Figs. 3, 4); (b) hypertrophy of the melanocytes and increased arborization (branching) of the dendrites of melanocytes; (c) augmentation of melanosome synthesis manifested by an increase in the number of melanosomes both in the melanocytes and in malpighian cells (keratinocytes) (Figs. 8, 9). The number of fully melanized melanosomes (stage IV) is increased both in the melanocytes and the associated pool of keratinocytes. Even the number of early and intermediate stage (partially melanized, stage I and II) melanosomes is increased; (d) an increase in tyrosinase activity due principally to the synthesis of new tyrosinase in the proliferating melanocytes; (e) an increase in the transfer of melanosomes from melanocytes to keratinocytes as the result of increased turnover of keratinocytes; (f) an increase in the size of melanosomes and also an increase in the size of the melanosome complex. This is, however, greatly dependent on the racial complexion and

genetic background of the individual. These observations are illustrated in Figs. 9 and 10 and summarized in Table 2.

Pigmentary responses in individuals who are exposed to sun can be grouped into the following five categories:

I. (Easy burn and no tan): People who sunburn very easily and do not show visually recognizable evidence of tanning (e.g., very fair skin, red hair, blue eyes, freckled skin, people with Celtic background—Irish and Scottish).

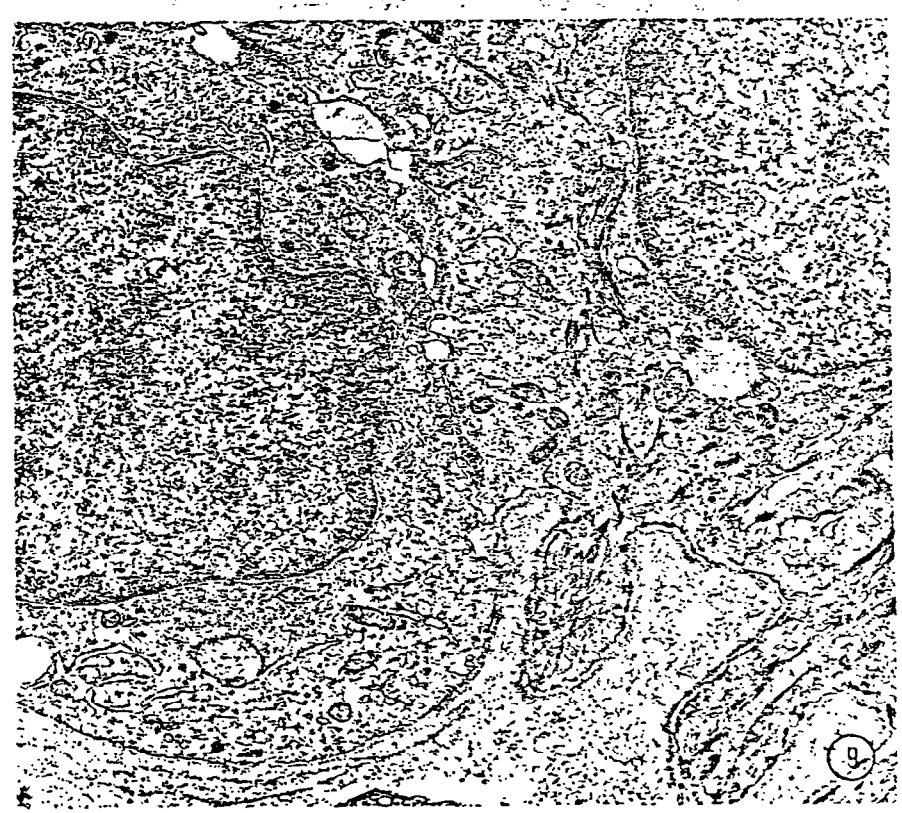

Fig. 9. An electron micrograph of a melanocyte and a keratinocyte from an unexposed Caucasoid back skin prior to UV irradiation. On the left side of the figure one can see a melanocyte with few melanosomes in stages II and III of their development. Notice the perinuclear distribution of 10-nm filaments. The keratinocyte (right side) shows few melanosomes which are in aggregated or complex forms.

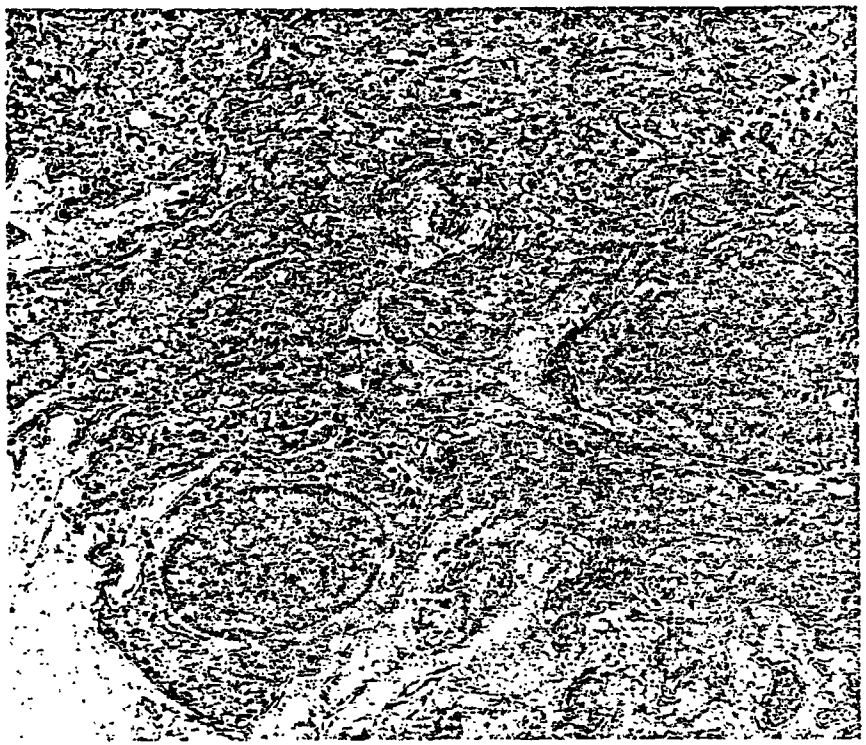

Fig. 10. An electron micrograph of a Caucasoid back skin during the delayed tanning (DT) reaction induced by UV-A radiation (315–400 nm). The specimen was taken from the same subject shown in Fig. 9. There are three melanocytes laden with numerous, highly melanized (stage IV) melanosomes. Note the increased number of transferred melanosomes in the keratinocytes. The melanosomes are either singly dispersed or are aggregated and forming complexes. Both UV-B (290–315 nm) and UV-A plus 8-MOP can induce similar changes (see Table 2).

II (Easy burn and slight tan): People who sunburn easily and tan slightly (e.g., moderately fair skin, people with blond hair, blue-green, or hazel eyes).

III. (Burn and then tan): People who burn moderately in the beginning, acquire a tan readily, and then do not generally burn on subsequent sun exposures (e.g., "brunette skin," olive skin, medium color skin).

TABLE 2. Changes in Epidermal Melanin Unit in Delayed Tanning Reaction after Repeated Exposures to UV-B, UV-A, or UV-A plus Oral 8-Methoxypsoralen (8-MOP)

Nature of observations	Nonexposed skin	Exposed skin after UV-B, UV-A, or UV-A plus oral 8-MOP
Macroscopic		
Degree of visual pigmentation	+	Increased with UV-B ++, UV-A +++, UV-A plus 8-MOP ++++;
Onset of visual pigmentation	Continuous at steady state	UV-A within 48 h UV-B within 72 h UV-A plus 8-MOP within 96-120 h
Degree of erythema reaction	None	Minimal with UV-A, moderate with UV-B, most with UV-A plus 8-MOP, but minimal with UV-A plus trimethylpsoralen.
Light microscopic		
Melanin granules	Barely visible in fair Caucasoids but easily visible in Negroids and Mongoloids	Increased in all races; most with UV-A plus 8-MOP
Number of melanocytes	About 700–950/mm² in unexposed skin and 1000–1300/mm² in habitually exposed skin; no racial differences in population density	Increased 2–3 times within 7–10 days; most remarkable increase in UV-A plus 8-MOP
Perikaryon of melanocytes	Small	Markedly enlarged
Dendrites of melanocytes	Poorly developed	Prominent and marked arborization
Dopa reaction	Weak	Strong; UV-A plus 8-MOP > UV-A; UV-A ≧ UV-B
Tyrosine reaction	Barely detectable or absent	Increased and easily detectable in all races
Electron microscopic		
In melanocytes:		
Number of melanosomes	Few	Markedly increased

Continued

TABLE 2. Continued.

Nature of observations	Nonexposed skin	Exposed skin after UV-B, UV-A, or UV-A plus oral 8-MOP
Melanization of melanosomes	Predominantly unmelanized in fair-skin (stages I–III); melanizing forms (stage III–IV) in dark skin	Melanized melanosomes (stages III and IV) markedly increased. In UV-A and UV-A plus 8-MOP most of the melanosomes are fully melanized; in UV-B melanosomes are in various stages of melanization
Size of melanosomes	Small (400–500 nm in long axis) Caucasoids; large in Negroids (500–700 nm in long axis)	Some increased; some are 700–800 nm even in Caucasoids
Distribution of melanosomes	Perinuclear and rarely in dendrites	Variable (diffused in perikaryon and dendrites); more prominent in dendrites particularly with UV-A or UV-A plus 8-MOP
10-nm (100 Å) filaments	Dense, perinuclear aggregation	Diffusely scattered in perikaryon and dendrites
Golgi apparatus	Poorly developed	Well developed, marked increase in size and number
Rough endoplasmic reticulum	Poorly developed	Well developed
Microtubules	Perinuclear	Perinuclear and in dendrites
In keratinocytes:		
Distribution pattern of melanosomes	Aggregated (melanosome complexes) in Caucasoids, nonaggregated in Negroids and Australoids, and mixed (aggregated and single) in Mongoloids.	Slightly altered (more single and aggregated melanosomes) in Caucasoids and Mongoloids; in Negroids nonaggregated form
Ratio of nonaggregated versus aggregated form of melanosomes	Predominantly aggregated in Caucasoids; nonaggregated in Negroids	Increased ratio of nonaggregated form, particularly with UV-A plus 8-MOP
Number of melanosomes per melanosome complex	5–6 melanosomes in Caucasoids	Fewer, 3–4 melanosomes in Caucasoids; most prominent decrease UV-A plus 8-MOP
Autophagic vacuoles in melanocytes and keratinocytes	Absent or very rare	Usually seen in UV-B and UV-A plus 8-MOP treated skin
Lipid droplets	Absent or very rare	Usually seen in UV-B and UV-A plus 8-MOP treated skin

TABLE 2. Continued.

IV. (No burn and good tan): People who do not burn readily but tan substantially; their eyes and hair are most likely dark (e.g., pigmented Caucasoids, Orientals, and others).

V. (Never burn and markedly tan): Markedly pigmented people (African and American Negroes, Australian Aborigines) who generally never burn but get profusely dark skinned after sun exposure.

3.3. Action Spectrum for Melanogenesis

Wavelengths shorter than 320 nm, which cause sunburn (erythema), are considered to stimulate melanogenesis or delayed tanning most effectively (Blum, 1955, 1959). The action spectrum for sunburn induced by exposure to sunlight has a maximum at 300–307 nm; that for sunburn induced by UV radiation from artificial sources has a maximum at 250–254 nm (8-h response). The erythema effectiveness gradually decreases at 270–280 nm (a distinct trough is seen at 280 nm due to absorption by proteins of the stratum corneum) and shows a distinct rise at 290–293 nm. The erythema effectiveness subsequently decreases rapidly at 313–320 nm). Wavelengths longer than 320 nm are weakly erythemogenic (Pathak and Epstein, 1972; Ying *et al.*, 1974). It requires nearly 800-1000 times more energy to produce a minimally perceptible erythema (20–30 J cm^{-2}) at 320–400 nm than is required to produce a similar degree of erythema reaction at 290–300 nm (about 20–30 mJ cm^{-2}). If the erythema reaction were directly related to melanogenesis, the findings discussed above would suggest that the maximum efficiency for melanogenesis should be at 250–254 nm, followed

by slightly less response at 290–315 nm, and long-wave UV and visible radiation would be least effective in the stimulation of melanogenesis. It is, in fact, quite the opposite. Germicidal radiation is significantly less melanogenic than UV-B (290–315 nm) or UV-A (315–400 nm) radiation. The pigmentation produced by 254 nm is less intense and of shorter duration than that produced by UV-B or UV-A radiation (Parrish *et al.*, 1972). Several hundred multiples of the minimal erythema dose (MED) exposures of UV-C radiation ($\lambda < 280$ nm) will not produce any blistering reaction nor any intense pigmentation response, while as little as 3–6 times the MED exposure to 290–315-nm radiation may cause an intense erythema and pigmentation reaction. For example, an exposure to 254-nm radiation equivalent to 30 × MED will produce a maximum of grade 2+ pink erythema response and a minimal tan (grade +, light brown tan). On the other hand, an exposure to 297-nm radiation equivalent to 2–5 × MED will produce a grade 2+ pink erythema response and a moderate tan (grade ++, medium brown tan). An exposure dose of 10 × MED at 297 nm can produce a grade +++, deep brown tan. In recent studies (Ying *et al.*, 1974; Jimbow *et al.*, 1974*a,b*, 1975*b*; Willis *et al.*, 1972), it was observed that UV-A (315–400 nm) was less erythemogenic and induced less intracellular degenerative change than UV-B. UV-A was found to be more effective in the induction of new melanogenesis than was UV-B. For a long time it has been generally believed, and more or less firmly stated in the dermatologic literature, that melanogenesis (DT) is optimally initiated by UV light of the so-called erythema spectrum (i.e., by UV-B). As early as 1962, Pathak *et al.*, showed that irradiation of human skin with long-wave UV radiation (UV-A), and to a limited extent visible light, would not only stimulate IT but also new melanogenesis. Subsequent studies by Langner and Kligman (1972) and Willis *et al.*, (1972) reemphasized the profound stimulation of melanin pigmentation by UV-A. In fact, UV-A appears to be more effective in the induction of new melanogenesis than UV-B radiation (Jimbow *et al.*, 1974*a,b*, 1975*b*). Thus, the generally held concept of the initiation of melanogenesis only by UV-B should be modified; long-wave UV light (UV-A) must be included in the melanogenic spectrum. It must be stressed, however, that if one were to estimate the quantum efficiency for melanogenesis by UV-B and UV-A, it would be apparent that UV-B is more efficient in the induction of melanogenesis than is UV-A. It requires approximately a minimum of 50–100 mJ cm^{-2} to stimulate melanogenesis by UV-B, whereas a minimum of 10–12 J cm^{-2} of UV-A is required to stimulate melanogenesis. It would appear, therefore, that the experimentally observed marked stimulation of melanogenesis by UV-A, both by Jimbow *et al.*, (1974*a,b*, 1975*b*) and Langner and Kligman (1972), is due to the fact that the less energetic photons associated with UV-A radiation (about 70–80 kCal mol^{-1}) cause less cellular degeneration than does UV-B (about 95–100 kCal mol^{-1}). Differences in the depth of transmission and absorption of UV-A and UV-B radiation within the epidermis are also important factors in the differential activation of the epidermal melanocytes. In fair-skinned individuals, most of the impinging UV-B radiation (about 75–80%) is absorbed by the nonviable multicellular layer of the stratum corneum. About 10–15% is absorbed by the viable cells of the malpighian layer (keratinocytes), and about 7–10% will transmit through the dermo–epidermal junction and be absorbed in the papillary dermis (Pathak and Epstein, 1971; Pathak and Fitzpatrick, 1974). It is the absorbed radiation at the dermo–epidermal junction where the melanocytes are localized that stimulates or activates melanogenesis. UV-A radiation, on the contrary, can penetrate deeper through the dermo–epidermal junction. Nearly 20–35% of the impinging radiation will penetrate through the epidermis and reach the hairbulb region. It appears that the better stimulation of melanogenesis by UV-A than by UV-B is due to the selective activation and proliferation of melanocytes both at dermo-eipdermal junction and in the hairbulb. Many of the ultrastructural changes reflecting formation, melanization, transfer, and degradation of melanosomes that take place in skin following repeated treatments with either UV-B, UV-A, or UV-A plus 8-MOP (8-methoxypsoralen) are listed in Table 2. For details concerning the stimulation of pigmentation by UV-A plus 8-methoxypsoralen, a potent photosensitizing agent of skin, the reader is referred to the articles by Pathak *et al.* (1974) and Pathak and Fitzpatrick (1974).

Visible radiation (400–700 nm) and infrared radiation ($\lambda > 750$ nm) are extremely weak in the induction or stimulation of melanogenesis. A single exposure in the range of 25–30 J cm^{-2} of visible light will not stimulate melanogenesis. Repeated exposures of skin to either visible radiation or to infrared radiation may stimulate some melanogenesis but most of the stimulation is secondary to the effects of heat resulting from prolonged absorption of the radiant energy.

3.4. Effect of Single and Multiple Exposures of UV Radiation on Human Melanocytes

Photobiologic processes that lead to hyperpigmentation of skin following a single exposure to UV-B or UV-A are different from those that result from multiple exposures to UV-B or UV-A radiation. The increased melanin pigmentation after a single exposure to either UV-B or UV-A radiation primarily reflects changes in the functional activity of the melanocytes, whereas the hyperpigmentation induced by multiple exposures

to either UV-B or UV-A reflects changes not only in the functional activity of the melanocytes but also the numerical changes in the epidermal melanin units (i.e., in the melanocytes and the associated pool of keratinocytes). A single exposure to either UV-B or UV-A causes none or minimal change in the number of functional melanocytes, but reveals an increment in the synthesis, melanization, and a transfer of melanosomes (Pathak *et al.*, 1965; Jimbow *et al.*, 1974*a,b*, 1975*b*). Multiple exposures to either UV-A or UV-B cause a marked increase in (a) the number of melanocytes; (b) the number of melanosomes synthesized; (c) the degree of melanization of the melanosomes as a result of an increase in tyrosinase activity; (d) the number of melanosomes transferred to the keratinocytes; (e) a distinct alteration in the size of some of the melanosomes (some of the newly synthesized melanosomes are larger in size than those in the unexposed skin); and (f) alteration in the distribution pattern of melanosomes in keratinocytes as a result of a change in the size of some of the melanosomes.

4. PHOTOPROTECTIVE ROLE OF MELANIN

In pigmented peoples, there exists a unique light absorbing and filtering system that minimizes the impact of photons on the vulnerable viable cells of the epidermis. The "white" population possesses a much less protective neutral density filter known as melanin, while the albino skin has virtually no neutral density filter, and more UV light penetrates such skin. In people with absence of melanin or in people who sunburn easily and tan poorly, skin will develop, early in life, abnormal changes caused by sunlight: wrinkling, keratoses, telangiectasia, and skin cancer (Fitzpatrick *et al.*, 1974). The onset of the cancerous or other abnormal changes is directly related to the degree of sun exposure and the latitude at which the person resides (i.e., sun intensity times the duration of exposure), and is inversely related to the amount of melanin in the skin. Among races with dark skin, in which melanin pigment effectively filters UV radiation, there is very little skin cancer. This photoprotective role of melanin is attributable to its presence in the particulate and nonparticulate form in the epidermis. In the stratum corneum most of the melanin is usually in the nonparticulate, amorphous form, although in certain individuals who are heavily pigmented, one does find a few melanosomes scattered randomly in the nonviable, horny cells. Most of the particulate forms of melanosomes are believed to be degraded due to the presence of hydrolytic activity associated with the melanosome complexes or in the outer membrane of the discrete organelles. Melanin present in the melanosomes is nondegradable (Hori *et al.*, 1968). The rest of the viable cells of the epidermis contain melanin-laden melanosomes in the particulate form.

The photoprotective role of melanin is accomplished by the following physical and chemical properties of the biochrome (Pathak and Fitzpatrick, 1974):

a. Melanin absorbs UV and visible radiation and can act as a neutral density filter. Melanins isolated from human hair, melanoma, and other biologic materials show high absorption without any characteristic peaks or absorption bands in the UV, visible, and near-infrared region (200–2400 nm). This absorption increases in the shorter wavelengths in the UV spectrum and appears to be due to highly conjugated system in the polymer.

b. Melanin-laden melanosomes attenuate the impinging radiation by scattering; this scattering involves any process that deflects electromagnetic radiation from a straight-line path and results in the attenuation of radiation. This increases the total absorbing path through which the UV radiation must pass. For particles with the dimensions of the order of wavelength of light in the UV spectrum (0.3 μm), the impinging light may be scattered according to the Rayleigh relation (scattering is inversely proportional to the fourth power of the incident light). Maximum scattering occurs when the wavelength of light approaches the diameter of the particle. For particles larger in size than the wavelength of the incident light (e.g., melanosomes which are 0.3–1.0 μm in size), the scattering relationship is quite complex, and more incident light will be scattered in the forward direction than in the backward direction.

c. Melanin absorbs the radiant energy in the UV and visible spectra and dissipates the absorbed energy as heat. In this regard it is of interest to point out the hypothesis of McGinnes and Proctor (1973) that melanin in the cell may serve as a device by which it may convert the energy of the excited states into heat by a phenomenon known as photon-photon conversion. This hypothesis implies that melanin polymer can act as an amorphous semiconductor in which coupling of phonons (i.e., vibrational modes of the melanin polymer) to its excited electronic states plays a role in the dissipation of energy absorbed from the impinging radiation.

d. Melanin can also utilize the absorbed energy and undergo immediate oxidation through the generation of semiquinonoid free radicals (Pathak and Fitzpatrick, 1974).

e. Melanin, as a stable free radical, with its ability for oxidation and reduction, can act as a biologic electron exchange polymer and minimize the impact of the impinging photons on the other vulnerable cell constituents (e.g., DNA) (Pathak and Fitzpatrick, 1974). The free radicals in melanin are quite stable and the unpaired electrons seem to be limited to localized regions of the polymer or are stabilized by a large number of resonance structures. Because of the unpaired electrons in melanin it may in effect serve as a one-dimensional semiconductor, where any bound protons

serve as electron traps. A free flow of charge in the form of electrons is then possible through the melanin (Longuet-Higgins, 1960; Pathak and Stratton, 1968; McGinnes *et al.*, 1974). It is known that UV irradiation increases the spin concentration in biological tissues such as human skin. Trapping of free radicals which could disrupt the metabolism of living cells is thus feasible in presence of stable free radicals in melanin polymer.

5. REFERENCES

Blum, H. F., 1955, Sunburn, *in: Radiation Biology* (A. Hollaender, ed.), Vol. II, *Ultraviolet and Related Radiations*, pp. 487–528, McGraw-Hill, New York.

Blum, H. F., 1959, *in: Carcinogenesis by Ultraviolet Light* Princeton University Press, Princeton, N.J.

Blois, M. S., Zahlan, A. B., and Maling, J. E., 1964, Electron-spin-resonance studies on melanin, *Biophys. J.* 4:471–490.

Duchon, J., Fitzpatrick, T. B , and Seiji, M., 1968, Melanin 1968: Some definitions and problems, *in: Year Book of Dermatology, 1967–1968 Series* (A. W. Kopf and R. Andrade, eds.), pp. 5–34, Year Book Medical Publishers, Chicago.

Fitzpatrick, T. B., 1965, Mammalian melanin biosynthesis, *Trans. St. John's Hosp. Derm. Soc* 51:1–25.

Fitzpatrick, T. B , and Breathnach, A. S., 1963, Das epidermale melanin-Einheit system, *Derm. Wochenschr.* 147:481–489

Fitzpatrick, T. B., Miyamoto, M., and Ishikawa, K., 1967, The evolution of concepts of melanin biology, *in: Advances in Biology of Skin*, Vol. VIII, *The Pigmentary System* (W. Montagna and F. Hu, eds.), pp. 1–30, Pergamon, Oxford.

Fitzpatrick, T. B , Hori, Y., Toda, K., Kinebuchi, S., and Szabo, G., 1971*a*, The mechanism of normal human melanin pigmentation and of some pigmentary disorders, *in: Biology of Normal and Abnormal Melanocytes* (T. Kawamura, T. B. Fitzpatrick, and M. Seiji, eds.), pp 369–401, University of Tokyo Press, Tokyo.

Fitzpatrick, T. B., Quevedo, C., Szabo, G., and Seiji, M., 1971*b*, Biology of melanin pigmentary system, *in: Dermatology in General Medicine* (T. B. Fitzpatrick, K. A. Arndt, W. H. Clark, Jr., A. Z. Eisen, E. J. Van Scott, and J. H. Vaughan, eds.), pp 117–146, McGraw-Hill, New York.

Fitzpatrick, T. B , Pathak, M. A. Harber, L. C., Seiji, M , and Kukita, A., 1974, An introduction to the problem of normal and abnormal responses of man's skin to solar radiation, *in: Sunlight and Man* (M A. Pathak, L. C. Harber, M. Seiji, and A. Kukita, eds.), pp. 3–14, University of Tokyo Press, Tokyo

Frenk, E., and Schellhorn, J P , 1969, Zur Morphologie der epidermalen Melaninеinheit, *Dermatologica* 139:271–277.

Gates, R. R., and Zimmerman, A. A., 1953, Comparison of skin color with melanin content, *J. Invest. Derm.* 21:339.

Hadley, M. E., and Quevedo, W. C., Jr , 1966, Vertebrate epidermal melanin unit, *Nature (Lond.)* 209:1334–1335.

Hempel, K., 1966, Investigation on the structure of melanin in malignant melanoma with H^3- and C^{14}-DOPA labeled at different positions, *in: Structure and Control of Melanocyte* (G. Della Porta and O. Muhlbock, eds), pp 162–175, Springer-Verlag, Berlin.

Hori, Y., Toda, K., Pathak, M. A., Clark, W. H., Jr., and Fitzpatrick, T. B., 1968, A fine-structure study of the human epidermal melanosome complex and its acid phosphatase activity, *J. Ultrastruct. Res.* 25:109–120.

Jimbow, K., and Fitzpatrick, T. B., 1974, Characterization of a new melanosomal structural component—the vesicular globular body—by conventional transmission, high voltage and scanning electron microscopy, *J. Ultrastruct. Res.* 48:269–283.

Jimbow, K., and Fitzpatrick, T. B., 1975, The role of 100 A filaments in the elongation of melanocytic dendrites and in the movement and transfer of melanosomes, *J. Cell Biol.* (in press).

Jimbow, K., and Kukita, A., 1971, Fine structure of pigment granules in the human hair bulb: ultrastructure of pigment granules, *in: Biology of Normal and Abnormal Melanocytes* (T. Kawamura, T. B. Fitzpatrick, and M. Seiji, eds.), pp. 171–194, University of Tokyo Press, Tokyo.

Jimbow, K., Takahashi, M., Sato, S., and Kukita, A., 1971, Ultrastructural studies of melanogenesis in melanocytes of normal human hair matrix, *J. Electronmicrosc.* 20:87–92.

Jimbow, K., Pathak, M. A., and Fitzpatrick, T. B., 1973, Effect of ultraviolet on the distribution pattern of microfilaments and microtubules and on the nucleus in human melanocytes, *Yale J. Biol. Med.* 46:411–426.

Jimbow, K., Pathak, M. A., Szabo, G., and Fitzpatrick, T. B., 1974*a*, Ultrastructural changes in human melanocytes after ultraviolet radiation, *in: Sunlight and Man* (M. A. Pathak, L. C. Harber, M. Seiji, and A. Kukita, eds.), pp. 195–215, University of Tokyo Press, Tokyo.

Jimbow, K., Kaidby, K. H., Pathak, M. A., Parrish, J. A., Kligman, A. L., and Fitzpatrick, T. B., 1974*b*, Melanin pigmentation stimulated by UV-B, UV-A and psoralen, *J. Invest. Derm.* 62:548.

Jimbow, K., Davison, P. F., Pathak, M. A. and Fitzpatrick, T. B., 1975*a*, Cytoplasmic filaments in melanocytes: Their nature and role in melanin pigmentation, *in: Pigment Cell* (V. Riley, ed.), Vol. 2, S. Karger, Basel.

Jimbow, K., Pathak, M. A., Kaidby, K. H., Parrish, J., Kligman, A. L., and Fitzpatrick, T. B., 1975*b*, Effect of UV-A, UV-B, and psoralen on *in vivo* human melanin pigmentation, *in: Pigment Cell* (V. Riley, ed.), Vol. 2, S. Karger, Basel (in press).

Klaus, S. N., 1969, Pigment transfer in mammalian epidermis, *Arch. Derm.* 100:756–762.

Langner, A., and Kligmen, A. M., 1972, Tanning without sunburn with aminobenzoic acid-type sunscreen, *Arch. Derm.* 106:338–343.

Longuet-Higgins, H. C., 1960, On the origin of free radical property of melanins, *Arch. Biochem. Biophys.* 86:231–232.

Mason, H. S., 1967, The structure of melanin, *in: Advances in Biology of Skin, The Pigmentary System* (W. Montagna and F. Hu, eds.), Vol. 8, pp. 293–312, Pergamon, Oxford.

Mason, H. S., Ingram, D. J. E., and Allen, B., 1960, The free radical property of melanins, *Arch. Biochem. Biophys.* 86:225–230.

McGinness, J. E., and Proctor, P. H., 1973, The importance of the fact that melanin is black, *J. Theor. Biol.* 39:677–678.

McGuinness, J., Corry, P., and Proctor, P., 1974, Amorphous semiconductor switching in melanins, *Science* 183:853–855.

Mishima, Y., 1967*a*, Cellular and subcellular activities in the ontology of nevocytic and melanocytic melanosomes, *in: Advances in Biology of Skin* (W. Montagna and F. Hu, eds.), Vol. 8, pp. 509–548, Pergamon, Oxford.

Mishima, Y., 1967*b*, Melanotic tumors *in: Ultrastructure of Normal and Abnormal Skin* (A. S. Zelickson, ed.), pp. 388–424, Lea and Febriger, Philadelphia.

Mitchell, R. E., 1963, The effect of prolonged solar radiation on melanocytes of human epidermis, *J. Invest. Derm.* 41:199–212.

Mitchell, R. E., 1968, The skin of the Australian Aborigine: A light and electron microscopical study, *Austral. J. Derm.* 9:314–320.

Mottaz, J. H., and Zelickson, A. S., 1967, Melanin transfer: A possible phagocytic process, *J. Invest Derm* 49:605–610.

Moyer, F. H., 1966, Genetic variations in the fine structure and ontogeny of mouse melanin granules, *Amer. Zoologist* 6:43–66.

Nicolaus, R. A., 1968, Melanins, *in: Chemistry of Natural Products Series* (E. Lederer, ed.), Hermann, Paris.

Olson, R. L., Gaylor, J., and Everett, M. A., 1973, Skin color, melanin, and erythema, *Arch Derm.* 108:541–544.

Pathak, M. A., 1967, Photobiology of melanogenesis: Biophysical aspects, *in: Advances of Biology of Skin; the Pigmentary System* (W. Montagna and F. Hu, eds.), Vol. 8, pp. 387–420. Pergamon, Oxford.

Pathak, M. A., and Epstein, J. H., 1971, Normal and abnormal reactions of man to light, *in: Dermatology in General Medicine* (T. B. Fitzpatrick, K. A. Arndt, W. H. Clark, Jr., A. Z. Eisen, E. J. Van Scott, and J. H. Vaughan, eds.), pp. 977–1036, McGraw-Hill, New York.

Pathak, M. A., and Fitzpatrick, T. B., 1974, The role of natural photoprotective agents in human skin, *in: Sunlight and Man* (M. A. Pathak, L. C. Harber, M. Seiji, and A. Kukita, eds.), pp. 725–765, University of Tokyo Press, Tokyo.

Pathak, M. A., and Stratton, K., 1968, A study of the free radicals in human skin before and after exposure to light, *Arch. Biochem. Biophys.* 123:468–476.

Pathak, M. A., and Stratton, K., 1969, Effects of ultraviolet and visible radiation and the production of free radicals in skin, *in: The Biologic Effects of Ultraviolet Radiation* (F. Urbach, ed.), pp. 207–222, Pergamon, Oxford.

Pathak, M. A., Riley, F. C., Fitzpatrick, T. B., and Curwen, W. L., 1962*a*, Melanin formation in human skin induced by long-wave ultraviolet and visible light, *Nature* (*Lond.*) 193:148–150.

Pathak, M. A., Riley, F. C., and Fitzpatrick, T. B., 1962*b*, Melanogenesis in human skin following exposure to long-wave ultraviolet and visible light, *J. Invest. Derm.* 39:435–443.

Pathak, M. A., Sinesi, S. J., and Szabo, G., 1965, The effect of a single dose of ultraviolet radiation on epidermal melanocytes, *J. Invest. Derm.* 45:520–528.

Pathak, M. A., Hori, Y., Szabo, G., and Fitzpatrick, T. B., 1971, The photobiology of melanin pigmentation in human skin, *in: Biology of Normal and Abnormal Melanocytes* (T. Kawamura, T. B. Fitzpatrick, and M. Seiji, eds.), pp. 149–169, University of Tokyo Press, Tokyo.

Pathak, M. A., Kramer, D. M., and Fitzpatrick, T. B., 1974, Photobiology and photochemistry of furocoumarins (psoralens), *in: Sunlight and Man* (M. A. Pathak, L. C. Harber, M. Seiji, and A. Kukita, eds.), pp. 335–368, University of Tokyo Press, Tokyo.

Quevedo, W. C., Fitzpatrick, T. B., Pathak, M. A. and Jimbow, K., 1974, Light and skin color, *in Sunlight and Man* (M. A. Pathak, L. C. Harber, M. Seiji, and A. Kukita, eds.), pp. 165–198, University of Tokyo Press, Tokyo.

Robson, N. C. and Swan, G. A., 1966, *in: Structure and Control of the Melanocyte* (G. Della Porta and O. Muhlboch, eds.), pp. 155, Springer Verlag, New York.

Seiji, M., Fitzpatrick, T. B., Simpson, R. T., and Birbeck, M. S. C., 1963*a*, Chemical composition and terminology of specialized organelles (melanosomes and melanin granules) in mammalian melanocytes, *Nature* 197:1082–1084.

Seiji, M., Shimao, K., Birbeck, M. S. C., and Fitzpatrick, T. B., 1963*b*, Subcellular localization of melanin biosynthesis, *in: The Pigment Cell: Molecular, Biological and Clinical Aspects* (V. Riley and J. G. Fortner, eds.), pp. 497–533, New York Academy of Sciences, New York.

Starrico, R. J., and Pinkus, H., 1957, Quantitative and qualitative data on the pigment cells of adult human epidermis, *J. Invest. Derm.* 28:33–45.

Stratton, K., and Pathak, M. A., 1968, Photoenhancement of the electron spin resonance signal from melanins, *Arch. Biochem. Biophys.* 123:477–483.

Swan, G. A., 1963, Chemical structure of melanins, *Ann. N. Y. Acad. Sci.* 100:1005–1016.

Swan, G. A., 1964, Some studies on the formation and structure of melanins *Rend. Accad. Sci. Fis. Mat. Napoli* 31:1–20.

Szabo, G., 1954, The number of melanocytes in human epidermis, *Br. Med. J.* 1:1016–1017.

Szabo, G., 1959, Quantitative histological investigations on the melanocyte system of the human epidermis, *in: Pigment Cell Biology* (M. Gordon, ed.), pp. 99–125, Academic, New York.

Szabo, G., 1967*a*, Photobiology of melanogenesis: Cytological aspects with special reference to differences in racial coloration, *in: Advances in Biology of Skin*, Vol. 8, *The Pigmentary System* (W. Montagna and F. Hu, eds.), pp. 379–396, Pergamon, Oxford.

Szabo, G. S., 1967*b*, The regional anatomy of the human integument with special reference to the distribution of hair follicles, sweat glands, and melanocytes, *Phil. Trans. B.* 252:447–485.

Szabo, G., Gerald, A. B., Pathak, M. A., and Fitzpatrick, T. B., 1969, Racial differences in the fate of melanosomes in human epidermis, *Nature* (*Lond.*) 222:1081–1082.

Toda, K., and Fitzpatrick, T. B., 1971, The origin of melanosomes, *in: Biology of Normal and Abnormal Melanocytes* (T. Kawamura, T. B. Fitzpatrick, and M. Seiji, eds.), pp. 265–278, University of Tokyo Press, Tokyo.

Toda, K., Pathak, M. A., Parrish, J. A., Fitzpatrick, T. B., and Quevedo, W. C., Jr., 1972, Alterations of racial differences in melanosomes distribution in human epidermis after exposure to ultraviolet light, *Nature New Biol.* 236:143–145.

Toda, K., Pathak, M. A., Fitzpatrick, T. B., Quevedo, W. C., Jr., Morikawa, F., and Nakayama, Y., 1973, Skin color: Its ultrastructure and its determining mechanism, *in: Pigment Cell*, Vol. 1, *Mechanism in Pigmentation* (V. Riley, ser. ed.; V. J. McGovern and P. Russel, eds.), pp. 61–81, Karger, Basel.

Willis, I., Kligman, A., and Epstein, J. H., 1972, Effects of long ultraviolet rays on human skin: Photoprotective or photoaugmentative? *J. Invest. Derm.* 59:416–420.

Wolff, K., and Konrad, K., 1971, Melanin pigmentation: an *in vivo* model for studies of melanosomes kinetics within keratinocytes, *Science* 174:1034–1035.

Wolff, K., Jimbow, K., and Fitzpatrick, T. B., 1974, Experimental pigment donation in vivo, *J. Ultrastruct. Res.* 47:400.

Ying, C. Y., Parrish, J. A., and Pathak, M. A., 1974, Additive erythemogenic effects of middle (280–320 nm) and long- (320–400 nm) wave ultraviolet light, *J. Invest. Derm.* 63:273–278.

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The Interaction of UVA and UVB in the Production of Threshold Erythema

BARRY S. PAUL, M.D., AND JOHN A. PARRISH, M.D.

Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, U.S.A.

A study was done to demonstrate quantitatively and graphically the way in which suberythemogenic doses of broadband UVA and UVB interact in producing a visible erythema. On the backs of fair-skinned human volunteers the minimal erythema dose (MED) was determined for polychromatic UVA and UVB. Increasing fractions of the UVA MED were given to sites already exposed to various fractions of the UVB MED resulting in sites exposed to various doses of both UVA and UVB. The entire experiment was repeated with the order of wavebands reversed. It was demonstrated that when UVA was followed by UVB an erythema was produced in those sites where the sum of the fractions was equal to one, an interaction termed photoaddition. When the UVA exposure followed the UVB, erythema was again predominantly noted in those sites demonstrating photoaddition. However, in the latter case, numerous sites of threshold erythema were noted where the sum of the fractions was greater than one. This is suggestive of photorecovery. No evidence of photoaugmentation was observed with either order of exposure.

Ultraviolet radiation of wavelengths 320–400 nm (UVA) requires at least 1,000 times more energy to produce delayed erythema of human skin than does ultraviolet radiation of wavelengths 290–320 nm (UVB) [1]. Yet, in many circumstances, UVA may make significant contributions to the production of erythema. The amount of solar UVA reaching the earth's surface may be several hundred times greater than the amount of UVB. Many sunscreens that effectively block or diminish the highly erythemogenic UVB do not block UVA thus permitting prolonged sun exposures and even higher UVA exposure doses. Late afternoon or early morning sun exposure, traditionally believed to be innocuous, contains a great amount of UVA, which may significantly contribute to sunburn incurred midday primarily by UVB. The quantitative manner in which UVA and UVB interact is important for other practical reasons such as developing safety standards for UV exposure, predicting the response to any radiation source based on consideration of the spectral power distribution and the human erythema action spectrum, and for calculating exposure doses in broadband UV phototherapy.

There are 3 possible ways by which suberythemogenic doses of UVA and UVB could interact to produce a threshold erythema: the sum of the fraction of the minimal erythema dose of UVA (MED_A) and the fraction of the minimal erythema dose of UVB (MED_B) can equal one, an interaction termed photoaddition; the sum can be less than one, an interaction called photoaugmentation; or the sum can be greater than one, an interaction called photoprotection or photorecovery. The literature on this subject has found proponents for all 3 of these phenomena [2–9]. Because of the fundamental importance of this question to photobiology, we performed an experiment designed to carefully quantitate and graphically demonstrate which of the above interactions indeed occur.

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Reprint requests to: John A. Parrish, M.D., Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts 02114.

MATERIALS AND METHODS

Subjects

Six healthy paid volunteer subjects participated in this study. All had Type I skin, one had widespread vitiligo and all testing was done on vitiligo patches. Informed consent was obtained from each subject.

Ultraviolet Sources

The UVA source was a 2,500 w xenon arc with a f/1.5 quartz condensing lens system. Radiation was filtered through 6 cm of an aqueous solution of a 7% $CuSO_4$ and 7% $CoSO_4$ in a quartz based chamber and through a Schott WG 335 (1 mm) filter. It was then projected onto the skin in a uniform (±10% circular field using a f/4 quartz lens and a UVA 45° dichroic mirror. Irradiance was 200 w/m^2. Spectral irradiance of this source is shown in Fig 1, as measured by an International Light IL 783 spectroradiometer. With this source the UVB irradiation was at least 5 orders of magnitude less than the UVA irradiation.

The UVB source was a 1.2 × 1.2 m planar bank of closely spaced fluorescent sunlamp bulbs (FS40 Westinghouse). The output of this source was measured by a cosine corrected International Light UVB detector having a spectral responsivity weighted similarly to the action spectrum for delayed erythema. It had a biologically effective irradiance equivalent to 5.5 w/m^2 at 290 nm. Uniformity was ±5%. Full spectral irradiance of this source is given in Fig 2 as measured by an IL 783 spectroradiometer. Essentially all effective erythema producing irradiation from the source is UVB.

Determination of the MED Dose

The MED (minimal erythema dose) for UVA and UVB was determined for each subject. A series of eight 1 cm diameter circles ranging from 40 J/cm^2 to 100 J/cm^2 of UVA and 15 mj/cm^2 to 40 mj/cm^2 of UVB were exposed in 15% increments on the lower back. The sites were observed at 24 hr. The lowest exposure dose which resulted in a minimal perceptible erythema was defined as the threshold dose or MED for UVA (MED_A) and UVB (MED_B).

Waveband Interaction

An aluminum template of forty-nine 1 cm-diameter circles in a 7 × 7 display (7 rows by 7 columns) was affixed to the lower back. The first column received no UVA. The others received 1/6 MED_A, 2/6 MED_A, 3/6 MED_A, 4/6 MED_A, 5/6 MED_A and 1 MED_A respectively. UVB was then administered to the rows. The first row received no UVB, the others received 1/6 MED_B, 2/6 MED_B, 3/6 MED_B, 4/6 MED_B, 5/6 MED_B, and 1 MED_B respectively in a grid-like fashion superimposed on the UVA exposures. Hence, each exposure site received a different combination of fractions of MED_A and MED_B with the first column receiving only UVB and the first row receiving only UVA (see Fig 3). Twenty-four hours later each of the 49 exposure sites was read visually by at least two observers, recorded and photographed. The same process was then repeated on the opposite side of the lower back except the order was reversed with the UVB being given first followed by the UVA.

RESULTS

MED

The average MED_B and MED_A for the 6 subjects was 22 (range 17–26) mj/cm^2 and 66 (range 46–80) J/cm^2 respectively.

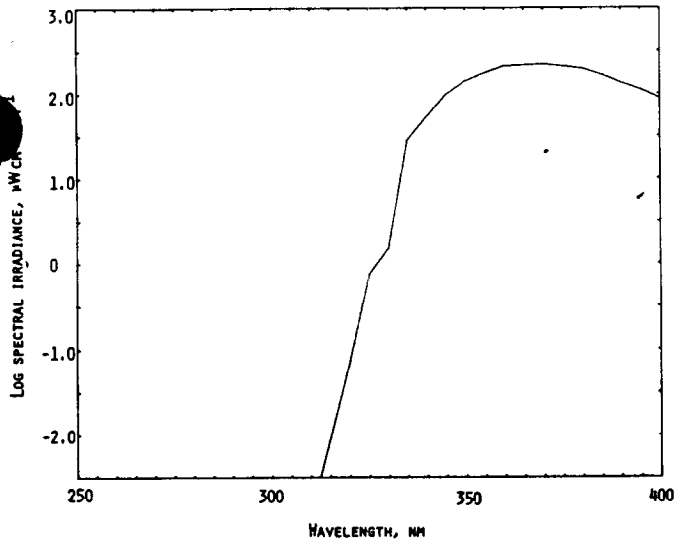

FIG 1. Spectral power distribution of UVA source (filtered xenon arc lamps).

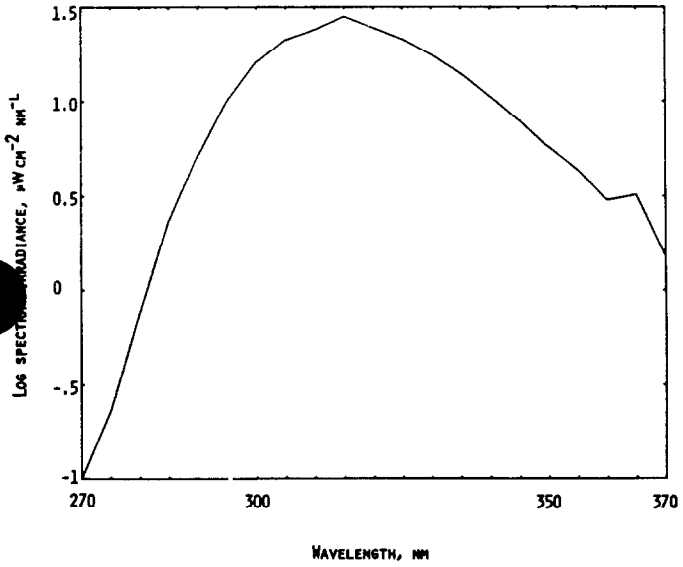

FIG 2. Spectral power distribution of UVB source (FS40).

Waveband Interaction

The first subject developed considerable immediate pigment darkening from the UVA exposure which persisted at 24 hr making it difficult to determine the erythema threshold points. Thereafter, only the lightest skin type I subjects and a subject with widespread vitiligo were exposed to minimize this effect. These 5 subjects demonstrated erythema threshold points predominantly in a line along the diagnonal in the exposures in which UVB was preceded by UVA (see Fig 4*A*). When the order was reversed with UVA being given after UVB the threshold points again occurred predominantly in a line along the diagonal although there was at least one instance in all 5 subjects where the erythema threshold point was located in the line above the diagonal (see Fig 4*B*). The sign test has been used to test the hypothesis that the median of all erythema threshold points occur on the diagonal line. The result shows that the occurrence of erythema threshold points in the line above the diagonal is not statistically significant ($p = 0.055$).

DISCUSSION

These results demonstrate that when suberythemogenic UVB is given after suberythemogenic UVA they interact by photoaddition in producing a threshold erythema. When the order is reversed and UVB is given first, the interaction again is predominantly photoaddition although there is a suggestion of mild photorecovery. No evidence of photoaugmentation was noted with either sequence of radiation.

To maximize the quantitative accuracy and interpretability

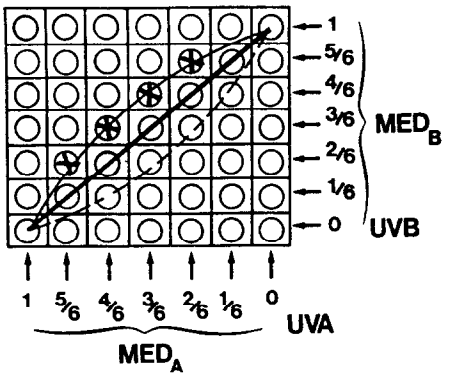

FIG 3. Experimental design. Each of the 49 exposure sites received a different combination of UVA and UVB. The *points* along the diagonal line from the lower left to the upper right are those which received sums of MED fractions equal to one (*solid line*). Threshold erythema occurring along this line is evidence for photoaddition. The *points* above or to the left of this line received a sum of greater than one and threshold erythema occurring in this area is evidence for photorecovery. One pattern of minimal photorecovery would be represented by (——). More striking photorecovery would place the threshold values further to the left upper portion of the design. The *points* below or to the right of the *solid diagonal line* received a combination totalling less than one, threshold erythema *points* in this region represent photoaugmentation. A pattern of minimal photoaugmentation is represented by (- - -).

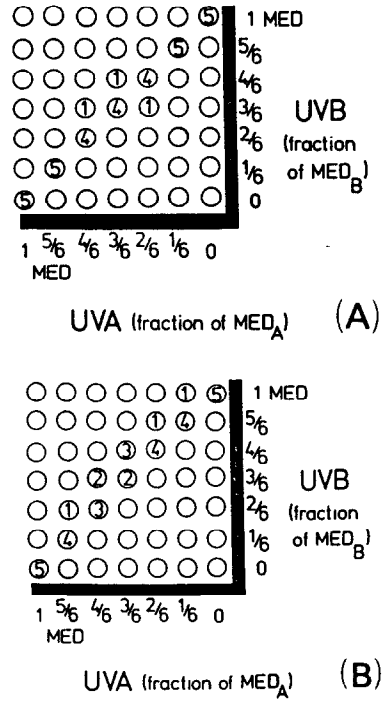

FIG 4. The encircled numbers represent how many of the 5 fair subjects demonstrated a minimal perceptible (threshold) erythema in that *circle*. All *empty circles* below these sites received less than threshold and demonstrated no erythema. *Empty circles* above received greater than threshold and showed erythema. Note that in Fig 4*A*, UVB following UVA exposure, the great predominance of threshold points are along the diagonal at the sites where the sum of the fractions are equal to one. In Fig 4*B*, UVA following UVB exposure, a number of threshold points occurred in the line above the diagonal.

his study, MED's for UVA and UVB were carefully deter-
ed in all subjects in the area immediately adjacent to the
erimental site. The experiment was carried out on the day
owing the MED determinations. While it is difficult to
urately differentiate gradations of erythema visually, the
ermination of the presence or absence of erythema is more
ise, repeatable and agreed upon by observers. In addition,
difference in dose-response curve for UVA and UVB ery-
ia, makes the interpretation of suprathreshold interactions
more difficult. Therefore, a minimal perceptible erythema
ised as the endpoint or threshold erythema.

he previous literature on this interaction is often difficult to
oret. van der Leun and Stoop [2] noted that 5 hr of indirect
ght filtered through windowglass increased by 20 to 30%
mount of previously given 250 nm and 300 nm radiation
ed to produce an MED. This demonstrates photorecovery
ecause they did not quantitate the UVA given in the five
exposure or calculate what percent this was of the MED_A,
egree of photorecovery is unknown. When they reversed
rder of exposures, giving the sunlight first, they report a
ase in the MED_{250} and MED_{300}. Similarly, it is not clear
er this was a photoadditive or photoaugmentative effect.
Veelden [3] also demonstrated photorecovery when UVA
iven following either UVB or UVC. Reversing the order
d photoaddition when UVC followed UVA exposure, and
photoaugmentation when UVB followed UVA. The pho-
mentation noted was not statistically significant. Willis,
an, and Epstein [4] claimed to observe photoaugmenta-
After their subjects were given a fixed dose of UVA, only
or 50% of the MED_B was needed to produce an erythema.
n this data is difficult to interpret because they did not
itate the MED_A in each subject. It is noteworthy that the
subjects whose MED_B was reduced the most had the lowest
al MED_B and therefore probably the lowest MED_A. Since
UVA exposures were fixed, one can infer that these subjects
ived a higher percentage of their MED_A. If it were as high
3/4 of the MED_A then the results could be explained by
otoaddition. Reversing the UV exposure order was not done
his study.

In a follow-up study Kaidbey and Kligman [5] combined
ctions of the determined MED_B to 10, 15 and 20 min of UVA.
Although they did mention that their source causes redness in
the average subject after 60 min of exposure, they did not
calculate the MED_A for each subject and so it is not known
what fraction of the MED_A was given. A study by Spiegel et al
[6] noted that 14 of 17 subjects demonstrated an erythema at
24 hr when 7.5 J/cm^2 of UVA was given before or after one-half
of the MED or UVB. Again the MED for UVA was not
determined. A similar problem arises in a recent study of Boer,
Schothorst, and Suurmond [7]. They noted that the MED_B was
reduced by 20% when combined with a prior irradiation of 10
J/cm^2 of UVA. They termed this photoaugmentation. However,
if the MED_A with their source was approximately 50 J/cm^2
their results would then be best described as photoaddition.
They did not reverse the order of exposure. Ying, Parrish, and
Pathak [8] found photoaddition to be the case for UVA and
UVB regardless of the order given. However, when UVB was
given prior to UVA, only one dose of UVA was used, combined
with fractions of the MED_B. With such limited exposure com-
binations it is doubtful that the mild photorecovery effect
observed in our study would have been discernible to them.
Sayre, Olson, and Everett [9] using a monochrometer showed
that combined exposures to wavelengths 254 nm, 280 nm, and
297 nm caused photoaddition for delayed erythema. Using 14
different combinations of these wavelengths, the average of the
sum of the fractions needed to produce a minimal erythema
as 0.98.

In the presented work, we are measuring the interaction of
suberythemal doses of UV. It is an important concept that even
at these suberythemal doses there are biological changes in the
skin. Studies have shown that the suberythemal doses of UVB
[10] and UVA [4] cause DNA damage, inducing an increase of
H^3-Thymidine sparsely labeled cells, which signifies DNA re-
pair. In both cases this DNA damage occurred at doses too
small to cause histologic changes or erythema. Suberythemal
doses of UVB can cause sunburn cell production [11]. Subery-
themal doses of UVA can cause delayed melanogenesis in
contrast to UVB which requires erythemal doses to produce
melanogenesis [12]. Finally suberythemal doses of both UVB
and UVA given at 24 hr intervals appear to have a cumulative
effect resulting in the lowering of the erythema threshold [12].
The photoadditive and mildly photoprotective interactions
herein described are only certain for the production of threshold
erythema. It has been suggested that UVA does not enhance
UVB-induced sunburn cell production [11]. Interactions be-
tween UVA and UVB in causing DNA damage or melanogen-
esis have not yet been well quantified for human skin.

When studying the interaction of UVA and UVB in the
production of erythema, one must remember that the skin's
biological response to UVA and UVB are different. UVB ery-
thema can be induced with about 20–50 mj/cm^2, peaks at 12–20
hr, has a modest dose-response curve, and is superficial, pink-
red and easily blanched. UVA erythema requires more than
1,000 times more energy, i.e. about 50–100 J/cm^2, may peak
sooner, has a steeper dose response curve, and is deep-red to
violaceous requiring firm pressure to blanch out. One possible
reason for these differences is that the erythemas are being
produced by different mechanisms. UVA and UVB may be
absorbed by different chromophores setting off a different chain
of events leading to erythema. Because of less epidermal ab-
sorption and less dermal scattering, UVA penetrates much
more deeply into the dermis than UVB [13]. Erythemogenic
doses of UVA cause primarily vessel damage and a dermal
infiltrate; equally erythemogenic doses of UVB cause mainly
epidermal changes such as dyskeratotic sunburn cells [14]. The
mediators may also be different as evidenced by the fact that
indomethacin will cause temporary blanching of a UVB-in-
duced erythema but not a UVA induced erythema [15]. This
implies that prostaglandine may mediate part of the UVB
erythema but not the UVA erythema. UVA as well as visible
light may also produce immediate pigment darkening (IPD).
This is believed to be due to the photooxidation of preformed
melanin and in general is greater in subjects with more facula-
tive or constitutive pigment [1]. The IPD that we noted was
often quite marked and persistent, eventually blending into the
delayed tanning. We noted no evidence that IPD is photopro-
tective.

No immediate erythema was noted with UVA or UVB. This
differs from a previous study [16] which noted significant im-
mediate erythema from UVA which persisted and blended into
delayed erythema with no diphasic response. This difference
may be related to the fact that the UVA irradiance in the above
study was considerably higher than ours.

The suggestion of photorecovery which occurs when UVA
exposure follows UVB is very interesting. It should be empha-
sized that this means that the UVB erythema may be increased
by subsequent UVA exposure, but not as much as would be
predicted by their addition. There is now evidence which sug-
gests that human epidermal cells contain photoreactivating
enzyme [17]. This enzyme in the presence of UVA or visible
light repairs thymine dimers which can be caused by prior UVB
exposure. The relationship, if any, of UV-induced DNA damage
and repair to erythema production is not known. However, it is
possible that UVA, by hastening DNA repair, lessens the ery-
thema response.

Although the mechanism and mediators of UVA and UVB
erythema production and their interaction are still largely un-
known, we have shown clinically that they interact predomi-
nantly via photoaddition to produce a threshold erythema.
Photorecovery is suggested in the case when UVA follows UVB
exposure and further studies are in progress to confirm this.
There was no evidence of photoaugmentation.

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REFERENCES

1. Parrish JA, Anderson RR, Urbach F, Pitts D: UV-A Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet. New York, Plenum Press, 1978
2. van der Leun JC, Stoop TH: Photorecovery of Ultraviolet Erythema, The Biological Effects of Ultraviolet Radiation (With Emphasis on the Skin. Edited by F Urbach). Oxford, Pergamon Press, 1969, pp 251–254
3. van Weelden H: Photorecovery in human skin, Lasers in Photomedicine and Photobiology. Edited by R. Pratesi, CA Sacchi. Berlin, Heidelberg, New York, Springer-Verlag, 1980, pp 129–133
4. Willis I, Kligman AM, Epstein J: Effects of long ultraviolet rays on human skin: Photoprotective or photoaugmentative? J Invest Dermatol 59:416–420, 1973
5. Kaidbey KH, Kligman AM: Further studies of photoaugmentation in humans: Phototoxic reactions. J Invest Dermatol 65:472–475, 1975
6. Spiegel H, Plewig G, Hofmann C, Braun-Falco O: Photoaugmentation. Arch Dermatol Res 261:189–200, 1978
7. Boer J, Schothorst AA, Suurmond D: Influence of UVA on the erythematogenic and therapeutic effects of UVB irradiation in psoriasis: photoaugmentation effects. J Invest Dermatol 76:56–58, 1981
8. Ying CY, Parrish JA, Pathak MA: Additive erythemogenic effects of middle (280–320 nm) and long (320–400 nm) wave ultraviolet light. J Invest Dermatol 63:273–278, 1974
9. Sayre RM, Olson RL, Everett MA: Quantitative studies on erythema. J Invest Dermatol 46:240–244, 1966
10. Gachnait F, Brenner W, Wolff K: Photoprotective effect of a psoralen-UVA-induced tan. Arch Dermatol Res 263:181–188, 1978
11. Kaidbey KH, Grove GL, Kligman AM: The influence of longwave ultraviolet radiation on sunburn cell production by UVB. J Invest Dermatol 73:243–245, 1979
12. Parrish JA, Zaynoun S, Anderson RR: Cumulative effects of repeated subthreshold doses of ultraviolet radiation. J Invest Dermatol 76:356–358, 1980
13. Anderson RR, Parrish JA: Optical properties of human skin, The Science of Photomedicine. Edited by JA Regan, JA Parrish. New York, Plenum Press, 1980, in press
14. Rosario R, Mark GJ, Parrish JA, Mihm, MC: Histological changes produced in skin by equally erythemogenic doses of UV-A, UV-B, UV-C and UV-A with psoralens. Br J Dermatol 101:299–308, 1979
15. Morison WL, Paul BS, Parrish JA: The effects of indomethacin on long-wave ultraviolet-induced delayed erythema. J Invest Dermatol 68:130–133, 1977
16. Kaidbey KH, Kligman AM: The acute effects of long-wave ultraviolet radiation on human skin. J Invest Dermatol 72:253–256, 1979
17. Sutherland BM, Harber L, Kochevar I: Pyrimidine dimer formation and repair in human skin. Cancer Res 40:3181–3185, 1980

Announcement

The Fifth Annual Westwood Carolina Conference on Clinical Dermatology will take place October 14–17, 1982, at the Hyatt at Palmetto Dunes, Hilton Head Island, South Carolina. For information and registration, contact Dermatology Educational Services, Post Office Box 4207, Kenmore, New York 14217, (716)884-1758.